

Research Article

Endothelial Function is impaired in Patients with Different Clinical Manifestations of Severe Cerebral Small Vessel Disease: Preliminary Results of SHEF-CSVD Study

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Abstract

Background: Endothelial functions in patients with different clinical manifestations of cerebral small vessel disease (CSVD) have not yet been thoroughly described.

Aim: The aim of the single center, prospective study being a part of SHEF-CSVD Study was to evaluate haemostatic factors and vascular reactivity reflecting endothelial function in different clinical manifestations of CSVD.

Methods: 107 patients with extensive MRI features of CSVD with recent lacunars stroke (LS), vascular Parkinsonism (VaP) or vascular dementia (VaD) and 32 controls (CG) matched for age and vascular risk factors were prospectively recruited. Blood markers of endothelial dysfunction and inflammation (thrombomodulin, uric acid, homocysteine and hs CRP), brachial artery flow-mediated dilatation (FMD) test were determined.

Results: Patients with CSVD compared to CG had significantly decreased FMD ($6,9 \pm 0,05\%$ vs $12,0 \pm 0,05$, $p < 0,001$); increased levels of hsCRP ($0,8 \pm 0,02$ vs $0,17 \pm 0,1$ mg/L; $p = 0,03$), homocysteine ($15,3 \pm 8,1$ vs $12,5 \pm 3,2$ $\mu\text{mol/L}$; $p = 0,04$) and thrombomodulin ($5,4 \pm 1,7$ vs $4,5 \pm 0,9$ ng/mL, $p = 0,009$). There was also a trend towards increased levels of uric acid in CSVD compared with CG ($5,9 \pm 1,7$ vs $5,4 \pm 1,1$ mg/dL, $p = 0,08$). No significant differences in analyzed factors were observed among LS, VaP and VaD patients.

Conclusion: Similar impairment of the cerebral microvasculature and endothelial function is present in different manifestations of CSVD compared to vascular risk factors matched control group.

Keywords: Cerebral small vessel disease; Endothelial dysfunction; Lacunar stroke; Vascular dementia; Vascular parkinsonism

Introduction

Cerebral small vessel disease (CSVD) is a syndrome of clinical, cognitive, neuro imaging and pathological findings resulting from brain damage in the cerebral white and deep gray matter [1]. Although it is associated with vascular risk factors like increasing age, hypertension and considered to result from cerebral arteriolar occlusive disease, the pathogenesis is largely unknown [2]. It probably starts with an increase in permeability of the blood-brain barrier caused by endothelial dysfunction. Endothelial cell dysfunction in CSVD can be assessed in vivo by measuring circulated molecules of endothelial origin (e.g. sP-selectin, thrombomodulin), molecules interacting with endothelial cells (e.g. hsCRP, homocysteine, uric acid) or by measuring systemic vascular function directly by means of forearm flow mediated dilatation (FMD). These markers are indeed usually altered in CSVD; however, these may simply reflect exposure to vascular risk factors [3]. Substantial endothelial dysfunction in common different clinical manifestations of CSVD such as lacunar stroke (LS), vascular Parkinsonism (VaD) and vascular dementia (VaD) have not been studied using standardized methodology. Available data regarding the role of endothelial activation in CSVD

is limited due to small sample size and/or without matched controls. We hypothesized that patients with different clinical manifestations of CSVD have higher levels of serum markers of endothelial dysfunction and altered peripheral vascular reactivity measured by FMD than controls.

Aim

The aim of the single center, prospective study was to evaluate endothelial functions by measuring haemostatic factors and vascular reactivity in different clinical manifestations of CSVD. It was conducted as a part of SHEF-CSVD Study (Significance of HE modynamic and haemostatic Factors in the course of different manifestations of Cerebral Small Vessel Disease) [4].

Methods

Patients with CSVD: with recent LS (n=25), established VaP (n=25), VaD (n=25) and 32 controls (CG) matched for age and vascular risk factors were prospectively recruited between January 2011 and January 2013 and hospitalized in Clinic of Neurology, Military Institute of Medicine, Warsaw, Poland. The study protocol and methods had been thoroughly described elsewhere [4]. In brief,

CSVD group consisted of patients fulfilling the following criteria: extensive CSVD features on MRI neuro imaging (described below), clinical symptoms of non-embolic lacunar stroke according to the Oxford Community Stroke Project or chronic vascular parkinsonism (after exclusion of other neurodegenerative conditions according to Hurtig scale) or vascular dementia (identified via the Modified Hachinski Ischemic Scale (score ≥ 7 points) and NINDS-AIREN (National Institute of Neurological Disorders and Stroke-Association International pour la Recherche et al. Enseignement en Neurosciences) criteria) [5-8]. Thirty two healthy subjects with normal MRI without signs of white matter lesions (WMLs) formed control group. Participants from both groups were aged between 60 and 90 years. Exclusion criteria comprised of: severe neurological deficits (mRS ≥ 3 points and/or NIHSS ≥ 10 points), altered consciousness, severe dementia (MMSE < 12 points), significant stenosis ($> 50\%$) of a major extra cranial or intracranial artery, atrial fibrillation, non-CSVD related WMLs (e.g. due to migraine, vasculitis). Clinical assessment (neurological, radiological, ultrasound) was performed by 2 "blinded" assessors unaware of the clinical diagnosis. This study was approved by the local Institutional Review Board and Ethics Committee and is supported by the Polish Ministry of Science and Higher Education as a research project of the Military Institute of Medicine (Warsaw, Poland, study number N N402 473840). All subjects were recruited after having signed the informed consent forms. The authors have no conflict of interest to declare.

Procedure

Vascular risk factor profiles were recorded for all participants. To prevent confounding by hyper acute phase responses, all LS patients underwent study procedures at least 2 weeks after their index strokes (mean 18 ± 3 days). We used 1.5 Tesla imaging with standard T2-weighted, FLAIR and gradient echo sequences. White-matter lesions were visualized on T2 and PD/FLAIR images as ill-defined hyper intensities ≥ 5 mm. The Fazekas scale was used to estimate the extent of the per ventricular and deep WMLs [9]. Extensive WML were defined as a score of 3 (per ventricular hyper intensities with involvement of white matter) on the perventricular scale, and/or a score of 3 on the deep white matter scale (beginning confluence of lesions or large confluent lesions). Generally accepted and widely available markers of endothelium damage and inflammation (thrombomodulin, uric acid, hs CRP) as well as arterial thrombosis and atherosclerosis

(homocysteine) were measured in all patients. Blood was sampled from the antecubital vein into 5-ml serum plasma tubes and was measured with commercially available ELISA kits according to the manufacturer's instructions (Bio Source, Europe, Nivelles, Belgium).

Ischemia-induced brachial artery flow dilatation which reflects NO-mediated endothelium-dependent vasodilator function was assessed according to standardized protocol [10]. This technique includes ultrasound evaluation of the brachial artery at rest (baseline), as well as during hyperaemia which was induced by a 5-minute inflation and deflation of a sphygmomanometer cuff placed around the forearm distally from the site of ultrasound measurement. Flow-mediated dilatation was estimated as the percentage increase in vessel diameter from baseline to maximum vasodilation, which occurred during hyperemia using visual inspection of single frames [11]. FMD was measured in the left arm except in subjects with a left-sided hemiplegia by experienced operators using a 10 MHz multi-frequency linear array probe attached to a high-resolution ultrasound (Loqic P6, GE Healthcare). To minimize the effect of confounding factors FMD was assessed when subjects were fasted and had avoided exercise, caffeine and medication intake for at least 6 h.

Statistical analysis

We compared levels of serum markers of endothelial dysfunction and FMD between patients with CSVD and CG assuming that the pathophysiology of CSVD manifestations is similar regardless of the underlying disease and also separately between LS, VaD and VaP groups. Log normal data were compared using paired tests; non-normal data were analyzed using non parametric tests. Homogeneity between groups was analyzed with the chi-square test for categorical and an analysis of variance for numerical variables. We also used evaluated unadjusted correlations using Spearman's rho between serum markers and FMD. We considered a p value < 0.05 to be statistically significant. All analyses were performed using Statistica 11 software (Stat Soft Inc, USA).

Results

One hundred and twenty patients were recruited. Of these, 13 subjects were excluded: 5 from CG group due to presence of asymptomatic signs of CSVD on MRI imaging, 8 from LS group due to the lack of extensive WMLs (Fazekas score < 3 points). Per

Table 1: Characteristics of lacunar stroke patients, vascular parkinsonism and vascular dementia patients and control subjects.

	Lacunar stroke patients (n=25)	Vascular parkinsonism patients (n=25)	Vascular dementia patients (n=25)	Control subjects (n=32)	X ²	p
Age, mean years (\pm SD)	69 \pm 4	70 \pm 5	72 \pm 4	69 \pm 5	1,21	0,38
Male sex	15 (65)	15 (65)	12 (48)	20 (63)	3,75	0,12
BMI, mean (\pm SD)	28 \pm 4	27 \pm 5	27 \pm 2	29 \pm 3	0,84	0,68
Hypertension	25 (100)	22 (88)	23 (92)	23 (72)	4,51	0,21
Coronary artery disease	15 (60)	11 (44)	14 (56)	18 (57)	1,47	0,46
Diabetes mellitus	15 (60)	15 (60)	11 (44)	14 (44)	1,76	0,51
Current smoking	12 (48)	8 (32)	9 (36)	10 (31)	5,30	0,15
Hypercholesterolemia	21 (84)	20 (80)	23(92)	29 (90)	1,89	0,59
Statin use	20 (80)	25 (100)	25 (100)	23 (72)	4,23	0,20
Antihypertensive use	24 (96)	22 (88)	23 (92)	23 (72)	3,99	0,18

Data are n (%) except where otherwise noted.

Table 2: Mean level of FMD, hsCRP, homocysteine, uric acid and thrombomodulin classified by study groups.

	Lacunar stroke patients (n=25)	Vascular parkinsonism patients (n=25)	Vascular dementia patients (n=25)	Control subjects (n=32)
FMD (%)	6,6±0,01***	6,0±0,05***	7,0±0,06**	12,0±0,05
hsCRP (mg/L)	0,51±0,7*	0,48±0,8*	1,06±1,7 **	0,17±0,1
Homocysteine (µmol/L)	14,9±5,7*	17,0±6,5***	13,9±4,3	12,5±3,2
Uric acid (mg/dL)	6,4±1,8*	6,2±1,4*	7,1±2,0*	5,4±1,1
Thrombomodulin (ng/mL)	5,4±1,8*	6,05±2,09**	5,25±1,6*	4,5±0,9

Data are means with SD. Statistically significant differences between CSVD and CG groups are indicated in bold.

Abbreviations: FMD: Flow-Mediated Dilatation; hsCRP: High Sensitivity C-Reactive Protein

*p<0,05; ** p<0,01; *** p<0,001

definition, none of the control subjects had WMLs or asymptomatic stroke. An overall of 107 patients (seventy five with CSVD and 32 with CG) fulfilled study criteria and were included to analysis. Vascular risk factor profiles and other patient characteristics were similar between CSVD and CG groups (Table 1). The overload of WMLs was analogous among CSVD groups (mean Fazekas score of 3). Patients with CSVD compared to CG had significantly decreased FMD (6,9±0,05% vs 12±0,05, p<0,001); increased hsCRP (0,8±0,02 vs 0,17±0,1 mg/L; p=0,03), homocysteine (15,3±8,1 vs 12,5±3,2 µmol/L; p=0,04) and thrombomodulin (5,4±1,7 vs 4,5±0,9 ng/mL, p=0,009). There was also a trend towards an increased level of uric acid in CSVD compared with CG (5,9±1,7 vs 5,4±1,1, p=0,08). Brachial flow mediated vasodilatation was significantly decreased and hsCRP, uric acid, thrombomodulin levels were increased in all separate CSVD groups while homocysteine level was increased in LS and VaD groups compared to CG (Table 2). No significant differences between CSVD cohorts with regards to mean FMD (H=0,77;p=0,68); hsCRP (H=0,24; p=0,88), homocysteine (H=5,49;p=0,07); uric acid (H=4,8;p=0,1) and thrombomodulin (H=2,27; p=0,32) were observed. All plasma markers of endothelial dysfunction inversely correlated with FMD and although they reached statistical significance (p<0,05) correlation coefficients were relatively low: Spearman rho ranged from -0,08 (uric acid) to -0,28 (thrombomoduline).

Discussion

This is the first study that has simultaneously evaluated brachial FMD and serum markers reflecting endothelial function in a well-phenotyped cohort of CSVD patients compared to control group. The main outcome of our study is that in comparison with controls matched for age, sex, arterial hypertension, diabetes mellitus, current smoking and coronary artery disease patients with clinically significant CSVD had higher levels of serum markers and weaker vascular reactivity, which indicates a more pronounced endothelial dysfunction. Furthermore, there was no significant difference in analyzed factors among patients with different clinical manifestations of severe CSVD indicating similar level of endothelial dysfunction in these states.

The pathophysiology of CSVD is complex and multi factorial. Main mechanisms hypothesized to be involved encompassing incomplete ischemia related to cerebral small vessel arteriolosclerosis and dysfunction of the cerebral endothelium leading to blood-brain barrier dysfunction, chronic edema and dysfunction of vasomotor reactivity and auto regulation [12]. Exposure to vascular risk factors, particularly hypertension and diabetes, probably initiate or accelerate endothelial damage, the inflammatory state and the procoagulant

and fibrinolytic activity. Our findings suggest that low-grade inflammation (as assessed by hsCRP) and endothelial activation (measured by thrombomodulin) are associated with cerebral micro structural disintegration or other pathologic changes in CSVD regardless of the different clinical manifestation. By measuring different soluble plasma markers (e.g. hsCRP, soluble intercellular adhesion molecule-1, soluble endothelial leukocyte adhesion molecule, thrombomodulin) previous studies have demonstrated their association with MRI white matter hyper intensities or diverse CSVD clinical manifestations [13-17]. Although homocysteine level in VaD did not differ from CG in our study (probably due to small sample size) we found that thrombomodulin, hsCRP and uric acid levels remained high in all CSVD groups in comparison to controls, which suggests their direct involvement in chronic endothelial activation or damage. Our findings stand in accordance with those obtained in the previous studies [18,19]. Combined measurement of these markers will undoubtedly add a higher degree of diagnostic accuracy to traditional CSVD risk assessment strategies. Our study also demonstrated a decreased response in FMD in CSVD which provided data regarding the impaired capacity of the endothelium to increase the bio-availability of NO. Similarly to our results, two studies assessing FMD in lacunar stroke patients showed an impaired response in FMD and L-arginine reactivity [20,21]. We also demonstrated a similar level of endothelial dysfunction in diverse manifestations of CSVD with comparable WMLs burden. The underlying mechanism is thus pathophysiological extensive, chronic and involves the whole vascular bed. However, the pathomechanism responsible for different clinical picture of CSVD remains unknown.

Our study has some limitations. The major weakness is the potential for random error or selection bias due to the small number of patients and controls included. The merit of our study lies in simultaneous estimation of two groups sharing similar risk factors and treatment profiles: selected patients with different clinical picture of CSVD and a well-matched control population.

Our study provides evidence for the involvement of endothelial dysfunction in different clinical manifestations of cerebral small vessel disease. Though mechanisms have yet to be determined and results of our study need confirmation in other populations, this could be a relevant contribution to pathophysiological concepts of CSVD that might lead to more effective therapies aiming at the reduction of CSVD burden especially in aging population.

Conclusion

Similar impairment of the cerebral microvasculature and

endothelial function is present in different manifestations of CSVD compared to vascular risk factors matched control group.

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